

particles from the media. After washing, 30×10^4 cells/ml were cultured in the presence of various concentrations of plant extracts in a humidified CO_2 incubator at 37°C . Uninfected Molt-4 cells were also cultured in the presence of similar concentrations of plant extracts. The number of viable cells were counted by the trypan blue exclusion method on days 2 and 4.

Figs 5, 6, 7, and 8 show that there were very slight reductions in Molt-4/HIV cell numbers as compared to uninfected Molt-4 cell numbers for GHX-2L, GHX-6L, and GHX-26F treated cultures. For each of the three treatments and the untreated control, there were increases in cell number relative to increases in days of culture. On the other hand, GHX-27L caused concentration dependent decreases in Molt-4/HIV cell number with little or no effect on uninfected Molt-4 cells. This indicates that one of the possible mechanisms of GHX-27L anti-HIV activity may be due to selective killing of HIV-infected cells. GHX-27L by selectively killing HIV chronically infected cells, should be able to inhibit indirect acute infection and cytopathicity and thus prevent CD4^+ lymphocytopenia in AIDS patients. It is very significant to note that up to date, no other drug or plant extract has been shown to be able to selectively kill HIV chronically infected cells.

8. Effects of plant extracts on virus production in HIV-1 chronically infected Molt 4 cells.

This example is presented to test whether any of the plant extracts will be able to inhibit HIV production in chronically infected cells and thus indirectly reduce HIV induced acute infection and cytopathicity. Supernatants from treated and untreated HIV-1 chronically infected Molt 4 cells (Molt-4/HIV) were titrated in MT-4 cells.

Figs 5, 6, 7, and 8 show that GHX-2L caused very significant concentration dependent reductions in virus yield with a concentration of 0.089 mg/ml causing >97% reduction in virus yield. GHX-26F was also significantly effective in reducing the

amount of virus production. GHX-6L and GHX-27L on the other hand, had only moderate effects on virus production from the chronically infected cells. Thus, GHX-2L and GHX-26F significantly inhibit late viral events and would inhibit indirect acute infection and cytopathicity and thus prevent CD4+ lymphocytopenia in AIDS patients. It should be noted that this property is an advantage over the classical nucleoside analogs like ddAzThd which do not affect HIV production from chronically infected cells.

9. Effects of plant extracts on HIV reverse transcriptase activity.

This example is presented to illustrate the effects of plant extracts on a key HIV induced enzyme that is essential in HIV acute infection. Inhibition of this enzyme would protect uninfected cells against HIV infections. The effects of plant extracts on reverse transcriptase (RT) activity in vitro were evaluated with enzyme from disrupted HTLV- IIIB particles.

RT assays were performed at 37°C for 60 min with 50 ul of a reaction mixture containing 50 mM Tris-HCl (pH 8.4), 2 mM dithiothreitol (DTT), 100 mM KCl, 10 mM MgCl₂, 0.01% Triton X-100, 1.25 uCi of [3H]-thymidine triphosphate, 50 ug/ml of poly(rA)-oligo(dT), 5 ul of test extract, and 5 ul of RT enzyme. The reaction was stopped by adding 5% trichloroacetic acid (TCA). Precipitates were then collected on glass fiber filters, washed with PBS, dried, and the radioactivity was measured in a liquid scintillation counter. The assays were carried out in triplicate.

All the four plant extracts tested (GHX-2L, GHX-6L, GHX-26F, and GHX-27L) showed concentration dependent reductions in RT activity (see Table 10). GHX-2L, GHX-6L, and GHX-26F caused 90% reduction of RT activity at concentrations between 0.013 and 0.020 mg/ml. On the other hand, GHX-27L was not able to cause 90% reduction in RT activity even at the highest concentration of 0.133 mg/ml tested.

10. Effects of plant extracts on Molt 4 clone 8 and M8166 cells.

The results of plant extract toxicity controls incorporated in examples 4 and 5 are hereby analysed in Figs. 9 and 10. Uninfected cells were exposed to various concentrations of plant extracts similar to what is described in 4. After 5 days of incubation, tetrazolium-based colorimetry was done on the cultures. The optical density (O.D.) values from treated wells without cells were subtracted from treated wells with cells. The resultant values were then expressed as percentages of control untreated wells with cells. These values were then plotted against the concentrations of the plant extracts (Figs. 9 and 10).

The EC50 values of the plant extracts against HIV-1 and 2 determined in examples 4 and 5, did not cause any adverse effects to the cells tested. The EC90 values caused minimal or no toxicity to the cells. From figures 9 and 10, the 50% cytotoxic concentrations (CC50) were determined and used in the calculation of antiviral indices in tables 4 and 5 which show that the plant extracts are indeed selective anti-HIV agents.

11. Ex vivo effects of plant extracts on peripheral blood mononuclear cells (PBMCs).

Accelerated death of PBMCs in HIV infected patients, particularly the CD4+ fraction, has been shown to be due to increased programmed cell death or apoptosis (Oyaizu et al, 1993). In vitro culturing of PBMCs enhances this phenomenon. Thus the ability of a drug or plant extract to slow down the accelerated death of cultured PBMCs may reflect their potentials in maintaining steady PBMC levels in HIV infected patients.

Figs 11, 12, 13, 14, and 15 show the effects of the plant extracts on culture induced cell death in the PBMCs of one healthy person and four AIDS patients. For comparison, ddCyd and ddIno were included in the tests. ddCyd, ddIno, and GHX-7L showed no